Estuarine, Coastal and Shelf Science 212 (2018) 365–371

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Response of estuarine meiofauna communities to shifts in spatial distribution of keystone species: An experimental approach

Monica Citadin[a,b], Tânia M. Costa[b], Sérgio A. Netto[a,*]

[a] Marine Science Laboratory, University of Southern Santa Catarina UNISUL, Av Acácio Moreira 787, Dehon, Tubarão, SC, 88704-900, Brazil
[b] Laboratory of Ecology and Animal Behaviour, São Paulo State University UNESP, Biosciences Institute, Coastal Campus, Praça Infante Dom Henrique, s/n, Bitaru, São Vicente, SP, 14 11330-900, Brazil

ARTICLE INFO

Keywords:
Range shifts
Fiddler crab
Meiofauna
Nematodes
Global warming

ABSTRACT

Current climate change directly affects species distribution by altering their physical environment and indirectly by altering interspecific interactions. The geographical distribution of fiddler crabs, keystone species of intertidal estuarine sediments, is supposed to expand poleward as a response to climate change. We experimentally investigate whether the introduction of a new species of fiddler crabs, where another different species already occurs, may affect the structural and functional composition of meiobenthic communities in intertidal areas. In order to disentangle the effect of abundance from species identity, we set up two indoor experiments (substitutive and a partial additive design) manipulating the diversity and density of two keystone species, *Leptuca uruguayensis* and *L. leptodactyla*. The results showed that the increase of the diversity keystone species did not impact any measured descriptors of nematode assemblages. By contrast, high density of keystone species, independent of the species, strongly affected the meiofauna total density, and the density of numerically dominant nematode genera. The results did not reveal any functional change in the meiofauna. Our experiments, designed to mimic the indirect effects of range expansion showed that while increasing diversity of functionally redundant keystone species had no effect on preys, increasing density negatively affected the structure of intertidal habitats.

1. Introduction

Current climate change is affecting marine biological processes at different scales, and impacting ecosystem services since it threatens the direct and indirect contributions that ecosystems make to human well-being (Brierley and Kingsford, 2009). Among other effects, ongoing warming has led to an increase in rainfall along the Southwestern Atlantic marine ecoregion (Bernardino et al., 2015), salinity changes in estuaries, and an increase in sedimentation in coastal areas (Robins et al., 2016). In the face of such change, species can respond by shifting their phenology or distribution to follow changing environments, by adapting to changing conditions in place, or, if unable to do either, by remaining in isolated pockets of unchanged environment (“refugia”) or, more likely, disappear (Holt, 1990; Parmesan and Yohe, 2003; Wiens et al., 2009; Crosby et al., 2016). In the marine realm, even though only a small portion of the many species introduced outside their native range of distribution may thrive, their effects can eventually be dramatic (Mack et al., 2000). However, the gradual shifts in physical conditions (e.g., temperature) have led species to settle in areas where now the conditions have become favorable (Walther et al., 2009; Burgiel and Muir, 2010).

Coastal areas of medium and high latitudes will likely face changes in biodiversity caused by the displacement of new species from adjacent regions due to climate warming (Parmesan and Yohe, 2003; Chen et al., 2011; Molinos et al., 2015). Based on ecological niche modeling, fiddler crabs, for example, are predicted to alter their distribution range, migrating poleward due to changes in temperature and precipitation patterns (Nabout, 2009). Along the Atlantic coast of South America, *Leptuca leptodactyla* (previously *Uca leptodactyla*) ranges from Venezuela to south Brazil, while the distribution of *L. uruguayensis* (previously *Uca uruguayensis*) ranges from Rio de Janeiro State to Mar Chiquita, Buenos Aires Province, north Argentina (Fig. 1) (Thurman et al., 2013). The southern part of Brazil (Laguna region, Fig. 1) is a biogeographic transition zone, the southern limit of mangroves on the American continent (Schaeffer-Novelli et al., 2000) and many tropical species of fiddler crabs (e.g., *L. leptodactyla*, occur up to the north of Laguna). At present, southwards of Laguna only L. *uruguayensis* and *Minuca mordax* (which inhabits areas with low salinity) occurs (Thurman et al., 2013).

Current global warming may directly affect some species,

* Corresponding author. Av Acácio Moreira 787, Dehon, Tubarão, SC, 88704, Brazil.
E-mail address: sergio.netto@unisul.br (S.A. Netto).

https://doi.org/10.1016/j.ecss.2018.07.025
Received 19 April 2017; Received in revised form 26 July 2018; Accepted 30 July 2018
Available online 01 August 2018
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Fig. 1. Distribution of the fiddler crab species *Leptuca uruguayensis* and *L. leptodactyla* (Thurman et al., 2013).

amplifying their distribution range, particularly in subtropical biogeographical transition zones (e.g., poleward expansion of mangroves, Cavanaugh et al., 2014). However, the responses by individual species and communities to climate change are not isolated process, but connected through indirect effects, as they may alter interspecific interactions such as predation and/or competition (Van der Putten et al., 2010; Classen et al., 2015). Thus, the functional identity of invasive species can influence the impact on native species and preys, with potentially higher impacts with greater niche diversity and differing functional identity, as invasive species complement one another (McCoy et al., 2012). But invasive species may have similar functional identity to a native species. Similar species may also negatively interact leading to a complete exclusion of the subordinate one, coexist with changes in abundance, behaviour or trophic level, as well as share impacts on preys (Russell et al., 2014). Niche differentiation of invasive similar species to native ones may occur quickly, suggesting that it is closely linked to the degree of competition (Hérault et al., 2008). Besides, as the species expand their distributions into areas of increased climatic suitability a decoupling of species interactions may allow some species to rapidly exploit a wider range of environments (Menéndez et al., 2008).

Leptuca leptodactyla and *L. uruguayensis* are morphologically similar species (Crane, 1975), have the same bioturbation potential (Machado et al., 2013; Natalio et al., 2017) and where they co-occur, often share the similar intertidal area (Ng et al., 2008; Checon and Costa, 2017). Fiddler crabs are conspicuous intertidal keystone species (*sensu* Power et al., 1996) from tropical and subtropical regions, and play a key role in controlling estuarine meiofauna by predation and changing the physical and chemical environment during their burrowing and feeding activities (e.g., Payton et al., 2002; Kristensen, 2008; Citadin et al., 2016). Due to the strong top-down regulation by fiddler crabs on the structuring and functioning of the intertidal meiobenthic community, the introduction of a new keystone species may increase the impact on the intertidal meiofauna communities. Here we hypothesize that, due to

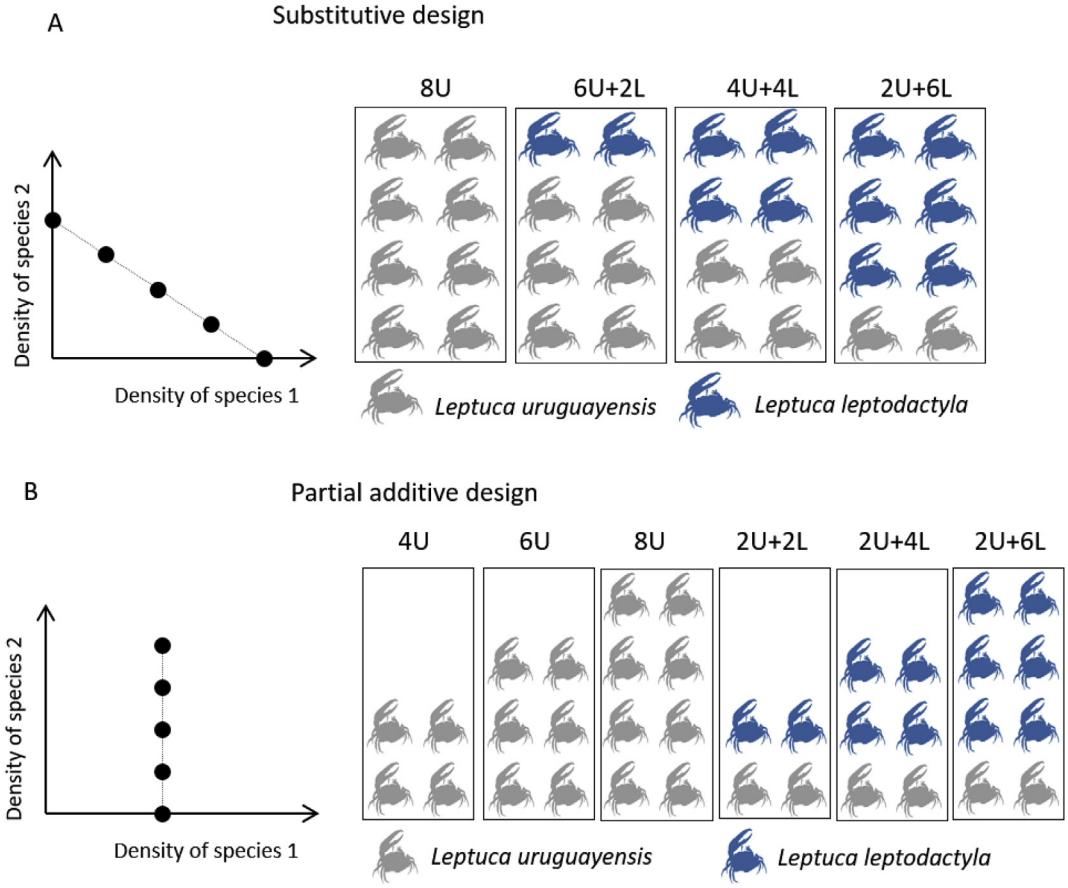

Fig. 2. Experimental setups used in this study: (A) in the substitutive design, the total density is constant while the proportion of each species changes; (B) in the partial additive design, with a single species density control, the total density varies as the density of one species is constant while the density of the other species varies. U: *Leptuca uruguayensis*; L: *L. leptodactyla*.

their high functional similarity, higher keystones species diversity may not increase the range of species preyed and change the direction and strength of meiofauna interactions; on the other hand, the increase of keystone species density, independent of crab species, may increase bioturbation and impose higher predation pressure, negatively affecting the meiofauna communities. To test these hypotheses, we designed laboratory experiments to mimic the effects of an invasion of *L. leptodactyla* to areas where only *L. uruguayensis* occur. Separate experiments were set up to cover two aspects of *L. leptodactyla* putative invasion: i-the change in diversity of keystone species (i.e. increased richness and evenness); ii-the increase in fiddler crab density.

2. Methods

We established two experimental setups to mimic the putative effects of the introduction of a new keystone species, where another single species already occurs, on meiofaunal communities. The experimental designs were based on the substitutive design (or replacement series) from De Wit (1960) and the partial additive design from Harper (1977) (Fig. 2). These designs differ in the way keystone species are manipulated. In the substitutive design, the total density of the crabs is held constant both in single and multiple-species treatments, while the proportion of each species changes (Fig. 2A).

In the partial additive design, the total density of crabs changes in single species treatment, and the density and proportion of species vary in the multiple-species treatment (Fig. 2B). The substitutive design was used to test the hypothesis whether an increase in keystone species richness and evenness affects meiofaunal communities, while the partial additive design was used to test the response of the meiofauna to changes in keystone species density.

2.1. Experimental conditions

Sediments (upper 5 cm layer) and fiddler crabs were collected on an unvegetated sandflat along the Una estuary, southeastern Brazil (24°24′50″S/47°04′14″W), within the Juréia-Itatins Reserve, which is part of a network of protected areas of Atlantic rainforest (Marques and Duleba, 2004). The crabs (only adult males of 8–10 mm of carapace width) were manually collected and transported to the laboratory in plastic boxes and placed in glass aquariums (29.5 × 11.5 × 20 cm, length x width x height) for acclimation to indoor conditions. We excluded females from the test because the period of reproduction (Benetti et al., 2007) when females can change their energy reserves and behaviour to benefit reproduction. The sediment, collected with a spade, was composed of very fine sand with an average total organic matter of < 2% and a chlorophyll *a* content ranging from 0 to 11.98 μg g^{-1} (Citadin et al., 2016). To set up the experiment, sediments were gently homogenized, and subsamples were taken to complete a layer of 5 cm of sediment. Fiddler crabs were placed in mesocosms only 48 h after the sediments to permit meiofauna stratification. The experiments were run at a constant temperature of 28 °C.

2.1.1. Set-up experiment 1: substitutive design

The substitutive design experiment was performed from October 27th to November 16th, 2014. The total density of keystone specimens was set at 8 inds/mesocosm (266 crabs/m^2). This density value is considered high, and was established considering the critical role played by competition in the manifestation of the niche partitioning effect (Checon and Costa, 2017). In this experiment, we manipulated the diversity of crabs, i.e. richness and the proportion of each species. The experiment included four treatments: one with a single keystone species and three with two keystone species. The single-species treatment (control) was composed of 8 *L. uruguayensis* individuals (8U), and the two-species treatments comprised: 6 *L. uruguayensis* + 2 *L. leptodactyla* (6U + 2L), 4 *L. uruguayensis* + 4 *L. leptodactyla* (4U + 4L), and 2 *L. uruguayensis* + 6 *L. leptodactyla* (2U + 6L) (Fig. 2A). Each treatment was replicated four times.

2.1.2. Set-up experiment 2: partial additive design

The partial additive design was performed from January 26th to February 15th, 2015. In this experiment, both keystone species density and diversity were manipulated. The density of *L. uruguayensis* was kept constant (two individuals) while an increasing density of *L. leptodactyla* was added to mimic the introduction of *L. leptodactyla* as an invasive species. The treatments were composed of: 2 *L. uruguayensis* + 2 *L. leptodactyla* (2U + 2L), 2 *L. uruguayensis* + 4 *L. leptodactyla* (2U + 4L), and 2 *L. uruguayensis* + 6 *L. leptodactyla* (2U + 6L). As a control, single-species treatments composed only of *L. uruguayensis* were included with the same levels of total density as the two-species treatment (i.e. 4U, 6U, and 8U) (Fig. 2B). Each treatment was replicated four times.

2.2. Sampling and sample processing

At the end of the experiments, crabs were counted and sediment samples were randomly collected from each mesocosm. A total area of 19.23 cm^2 of sediment was taken from each mesocosm (two PVC corers of 3.5 cm in diameter to a depth 1 cm, pooled). All sediment samples were fixed in 4% formalin and processed following Somerfield et al. (2005). The sediment was washed with fresh water and sieved through 500 and 63 μm mesh openings. The fauna retained by the smaller mesh was extracted by flotation in Ludox TM-50 (specific gravity of 1.15). The extracted fauna was placed in embryo dishes with glycerol (65% water, 30% alcohol and 5% glycerin), left to evaporate for 10 h, and then mounted on permanent slides.

2.3. Data analysis

At the end of the experiment, two replicates of experiment 2 (one from the 2U + 4L treatment and one from the 2U + 6L treatment) contained dead crabs and were therefore excluded from the statistical analysis. Only nematodes were used for data analysis as they accounted for 97% of the total meiofauna. Both univariate and multivariate statistical methods were used to test the effects of fiddler crab density and/or diversity on the nematode community.

For the univariate analyses both structural and functional descriptors were used. Structural descriptors included number of genera, total density, the density of numerically dominant genera, and diversity as the estimated number of genera (Hurlbert Index, ESn) (Hurlbert, 1971). Based on rarefaction techniques, the ESn is less dependent on sample size (Soetaert and Heip, 1990). As functional attributes of the nematode assemblages, the index of trophic diversity (ITD) and the maturity index (MI) were used. The index of trophic diversity (Heip et al., 1985) is based on the proportion of each of the four feeding types (selective deposit feeders, nonselective deposit feeders, epigrowth feeders and predators/omnivores) (Wieser, 1953). ITD values range from 0.25 (highest trophic diversity with the four trophic groups accounting for 25% each) to 1.0 (lowest trophic diversity when only one feeding type is present). The MI, derived from life history characteristics of nematode genera, was calculated for each sample according to Bongers et al. (1991, 1995). Nematodes were classified along a scale of 1–5, with colonizers (*inter alia* short life cycle, high reproduction rates, high tolerance to disturbance) classified as 1 and persisters (*inter alia* long-life cycles, few offspring, sensitive to disturbance) classified as 5.

For the first experiment (substitutive design), univariate one-way PERMANOVA tests (Anderson et al., 2008) were run on Euclidean distance matrices with 999 permutations and with unrestricted permutation of raw data. For the second experiment (partial additive design), two-way PERMANOVA tests were used to assess differences in the univariate and multivariate faunal structure among keystone species densities (fixed factor with three levels: 4, 6, and 8 crabs) and richness (fixed factor with two levels: *L. uruguayensis* and *L. uruguayensis* + *L. leptodactyla*) and interactions. Univariate PERMANOVA tests were run

on Euclidean distance, and multivariate on Bray-Curtis similarity matrices, both with 999 permutations of residuals within a reduced model and Type III sums of squares to cope with the unbalanced design (Anderson et al., 2008). In the case of significant differences ($p < 0.05$), pairwise tests based on Monte Carlo (MC) were applied. The PERMDISP (Anderson et al., 2008) technique was used to test the homogeneity of multivariate dispersions. Ordinations of the multivariate faunal data based on the genus-abundance matrix were represented by non-metric multidimensional scaling (nMDS).

3. Results

3.1. Experiment 1: substitutive design

In the substitutive design experiment, nematodes accounted for 97% of the total meiofauna. Nematode densities ranged from 36 to 126 inds/10 cm^2, consisting of 30 genera belonging to 14 families (a complete list in given in Appendix A). *Anoplostoma* (Anoplostomatidae) and *Microlaimus* (Microlaimidae) were the numerically dominant genera accounting for 25,1% and 19,9%, respectively, of the total nematodes.

There was no clear pattern of sample aggregation indicating that the structure of nematode assemblages did not differ among the treatments (nMDS, Fig. 3). The results of the multivariate PERMANOVA confirmed the absence of significant differences among treatments (p (MC) = 0,263; Table 1). All the structural and functional univariate descriptors of the nematode assemblage did not vary significantly with the increase in keystone species diversity (Table 1).

3.2. Experiment 2: partial additive design

As in the substitutive experiment, nematodes were the dominant meiofaunal group and accounted for more than 95% of the total fauna. Nematode densities ranged from 19 to 64 inds/10 cm^2, consisting of 20 genera belonging to 14 families (Appendix B). *Microlaimus* (Microlaimidae) was the numerically dominant genus accounting for 82.1% of the nematodes.

The nematode assemblage structure was clearly different between the crab density levels of 4 and 8 individuals, while samples taken in the 6-crab-treatments showed an intermediate nematode community structure (nMDS, Fig. 4). The multivariate PERMANOVA revealed that the nematode assemblages varied significantly among the different keystone species density levels (p (MC) = 0,016; Table 2), but was not affected by keystone species diversity (p (MC) = 0,135). The pair-wise tests confirmed the significant differences between nematode assemblages in the 4- and 8-crab-treatments, but not between treatments 4 × 6 (Appendix C).

The total nematode density and the *Microlaimus* density were significantly affected by the increase density of the combined keystone species (Fig. 5, Table 2). The highest crab density (i.e. 8 crabs) led to a significantly lower total nematode density compared to lower crab densities (i.e. 6 and 4 crabs) (Fig. 5A; Appendix C). *Microlaimus* densities decreased proportionally to the increased fiddler crab density with significant differences between the highest and lowest crab density levels (Fig. 5B and C, Appendix C). All the other descriptors of nematode assemblage structure did not vary significantly with the increase of fiddler crab density nor diversity (all p (MC) > 0,05; Table 2).

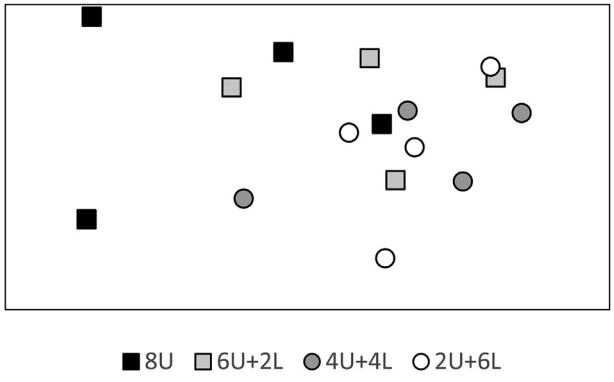

Fig. 3. MDS ordination of log (x + 1) transformed nematode genus abundances. Black squares: 8U; light gray squares: 6U + 2L; dark gray circles: 4U + 4L; open circles: 2U + 6L. U: *Leptuca uruguayensis*; L: *L. leptodactyla*. Stress 0.015.

Table 1
Results of PERMANOVA of the substitutive design, evaluating the effect of keystone species diversity on the multivariate structure and on structural and functional univariate descriptors of nematodes. df: degrees of freedom; SS: sum of squares; MS: mean squares; p (MC): p-value obtained with Monte Carlo permutation test.

Source of variation	df	SS	MS	F	p (MC)
Multivariate structure					
Treatment	3	1152.8	384.26	1.2007	0.263
Residual	12	3840.4	320.04		
Density					
Treatment	3	3303.7	1101.2	1.5694	0.253
Residual	12	8420.1	701.68		
Number of genera					
Treatment	3	3.414	1.118	1.3402	0.317
Residual	12	10.19	0.849		
ES (51)					
Treatment	3	0.0151	0.0050	0.8040	0.526
Residual	12	0.0753	0.0062		
Anoplostoma density					
Treatment	3	155.83	51.943	0.7309	0.575
Residual	12	852.77	71.065		
Microlaimus density					
Treatment	3	278.87	92.957	1.5802	0.243
Residual	12	705.94	58.828		
Index of Trophic Diversity					
Treatment	3	0.0030	0.0010	2.0308	0.158
Residual	12	0.0059	0.0004		
Maturity Index					
Treatment	3	0.0069	0.0023	0.2092	0.872
Residual	12	0.1321	0.0110		

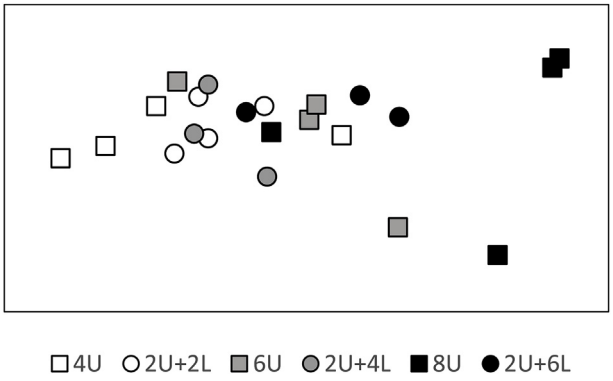

Fig. 4. MDS ordination of log (x + 1) transformed nematode genus abundances at densities of 4 (U: open squares; U + L: open circles), 6 (U: gray squares; U + L: gray circles), and 8 (U: black squares; U + L: black circles). U: *Leptuca uruguayensis*, L: *L. leptodactyla*. Stress 0.018.

4. Discussion

Climate change directly affects species by altering their physical environment and indirectly by altering interspecific interactions, such as predation and competition (Adler et al., 2009; Traill et al., 2010). Our study aimed at investigating if the introduction of a new keystone species where another single species already occurs affected intertidal areas through the interaction with meiofaunal communities. We experimentally mimicked the invasion of the fiddler crab *L. leptodactyla* to

Table 2
Results of two-way PERMANOVA of the partial additive design, evaluating the effect of keystone species density, richness, and interaction (density x richness) on the multivariate structural and functional descriptors of nematode assemblages. Bold values indicate $p \leq 0.05$. The results of the pairwise tests are shown in Table S3. df: degrees of freedom; SS: sum of squares; MS: mean squares; p (MC): p-value obtained with Monte Carlo permutation test.

Source of variation	df	SS	MS	Pseudo-F	p (MC)
Multivariate structure					
Fiddler crab density	2	1940.2	970.1	4.055	**0.006**
Fiddler crab richness	1	451.85	451.85	1.889	0.15
Richness x density	2	568.8	284.4	1.188	0.326
Residual	16	3827.3	239.2		
Density					
Fiddler crab density	2	1025.7	512.83	6.45	**0.01**
Fiddler crab richness	1	54.22	54.22	0.689	0.419
Richness x density	2	261.48	130.74	1.64	0.212
Residual	16	1270.5	79.40		
Number of genera					
Fiddler crab density	2	13.452	6.725	1.924	0.16
Fiddler crab richness	1	0.5041	0.5041	0.144	0.718
Richness x density	2	1.519	0.759	0.217	0.823
Residual	16	55.917	3.494		
Diversity ES (51)					
Fiddler crab density	2	0.9942	0.4971	1.185	0.341
Fiddler crab richness	1	0.3340	0.3340	0.796	0.382
Richness x density	2	2.1105	1.0552	2.516	0.365
Residual	16	6.7091	0.4193		
Microlaimus density					
Fiddler crab density	2	0.8126	0.4063	6.178	**0.012**
Fiddler crab richness	1	0.1130	0.1130	1.719	0.204
Richness x density	2	0.1909	0.0954	1.458	0.24
Residual	16	1.0522	0.0657		
Index of Trophic diversity					
Fiddler crab density	2	0.002911	0.00291	0.445	0.535
Fiddler crab richness	1	0.0158	0.0079	1.21	0.347
Richness x density	2	0.00912	0.00456	0.69	0.505
Residual	16	0.1044	0.00652		
Maturity index					
Fiddler crab density	2	0.00177	0.00023	0.178	0.832
Fiddler crab richness	1	0.00023	0.00089	0.046	0.835
Richness x density	2	0.0129	0.00645	1.292	0.312
Residual	16	0.0799	0.00499		

areas inhabited by *L. uruguayensis*. These species are functionally very similar, and we hypothesized that: 1) increasing keystone species richness and evenness would not increase the range of meiobenthic species preyed and change the direction and strength of meiofaunal interactions and community structure; 2) increasing keystone species densities, independent of the species, would increase bioturbation and impose higher predation pressure on the meiofauna. Our results showed that the increase of functionally similar keystone species diversity did not affect any measured descriptors of nematode assemblages. By contrast, high keystone species density negatively affected the meiofauna in terms of total density, diversity and the density of numerically dominant nematode genera.

Two major mechanisms are known by which predator diversity enhances resource capture: the selection effect (Huston, 1997) and the complementarity effect (Loreau, 2000; Ives et al., 2005; Griffin et al., 2008). In the former, also known as sampling effect (Huston, 1997), trait variation comes into play in an initial condition, as a higher predator diversity allows sampling of a wider trait range, and then a selective process promotes dominance by species with particular trait values. In the latter, the niche complementarity is evident, and species differing in resource requirements exploit a wider spectrum of resources and experience reduced interspecific competition. Our results showed that the two keystone species used in the experiment did not showed niche differentiation or partitioning of resources, as no increased consumption of a broad spectrum of nematode genera or total number of organisms was observed with increased fiddler crab diversity (richness and evenness). The functional redundancy of *L. leptodactyla* and *L. uruguayensis* provides a possible mechanistic explanation of absence of effects on meiofauna, and supports Griffin et al. (2008) contention that resource partitioning results from the effect of functional diversity rather than species richness *per se*. The functional redundancy (whether the species share similar biological traits) of *L. leptodactyla* and *L. uruguayensis*, does not imply that species are identical, as they may differ in their response to a variety of environmental factors (ecological redundancy) and this response diversity may promote resilience of the group as a whole to various kinds of shocks and fluctuations (Scheffer et al., 2015).

As observed with *L. leptodactyla* and *L. uruguayensis* in this study, and most likely with other species in the near future, range expansion in response to current global warming will lead to functional redundancy as primordial community functions would be fulfilled in priority for an assemblage to gain its basic ecological structure (Guillemot et al., 2011). Still, further laboratory and field experimental studies should investigate the indirect effects of expansion range in order to disentangle the effect of abundance from species identity, as one species may be functionally redundant in one situation but may become pivotal in another. Moreover, in contrast with larger field experiment, limited resource heterogeneity could preclude resource partitioning (Comte et al., 2016).

Increased fiddler crab densities, independent of species number, significantly affected the structure of the nematode assemblages. The total nematode density and the density of the most abundant genus (*Microlaimus*) decreased with increasing fiddler crab density. Fiddler crabs modulate meiofauna communities in different ways. Whereas feeding activity strongly reduce meiofauna abundances in superficial sediments (Hoffman et al., 1984; Reinsel, 2004), burrows and excavation pellets increase densities and diversity of meiofaunal assemblages (Citadin et al., 2016). During engineering activities of burrow construction and maintenance, fiddler crabs transport pellets of subsurface

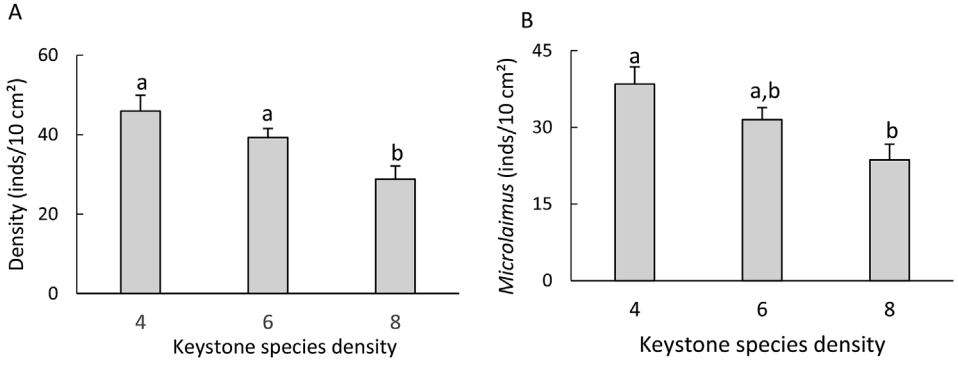

Fig. 5. Mean values (± SE) of nematode densities (A), and *Microlaimus* density (B) at densities of 4, 6, and 8 fiddler crabs (combined species). Different letters indicate significant differences after pairwise PERMANOVA tests.

sediments with meiofauna to the surface, concentrating the fauna from different sediment layers (Citadin et al., 2016). Both L. *leptodactyla* and *L. uruguayensis* have similar bioturbation potential (Natalio et al., 2017). Our results showed that, independent of the species analyzed in this experiment, the overall effect of increasing density of fiddler crabs was to depress nematode assemblages by more than 50%.

The inference about future climate changes relies on many different approaches, and a variety of experimental designs have been used to investigate their direct impacts on meiofauna (see revision by Zeppilli et al., 2015), such as on ocean warming and acidification (e.g., Gingold et al., 2013; Lee et al., 2017), hypoxia (e.g., Grego et al., 2013). On the other hand, experimental studies looking at the indirect impacts on benthic organisms are scarce so far. In this study, we apply two parallel designs with different assumptions about how expansion range affects meiofaunal communities. Whereas in the substitutive design the proportion of keystone species is density-independent, in the additive design the density of fiddler crabs is a co-variate of richness. Both experiments did not reveal any functional change in the benthic fauna due to indirect effects of range expansion.

Current global warming has important consequences for species distribution and for the structuring processes of communities. While the range of some species expands, contracts, or shifts as they adjust their geographic distribution (Parmesan and Yohe, 2003), the lack of knowledge about their invasive strategies and functional roles in the new habitat constitute a challenge for managing and conservation. We showed that while increasing diversity of functionally redundant keystone species had no effect on preys, increasing density negatively affected the structure of intertidal habitats, as indicated by detected changes in meiofauna assemblages.

Acknowledgements

We received full logistic support of the Juréia-Itatins Reserve, Peruibe-SP, Brazil. We thank B. Fogo, F.H.C. Sanches, F.R. DeGrande, L.F. Natálio, R. Carvalho, P.J. Jimenez, P. Granado and J. Pardo for their help during fieldwork. R. Christofoletti and Fabi Gallucci are thanked for the helpful comments on earlier versions of the manuscript. Funding: Monica Citadin was supported by the CAPES foundation (Ministry of Education), and Tânia Costa and Sérgio Netto by the Brazilian National Research Council (CNPq).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ecss.2018.07.025.

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